TAR CANCER IN MICE: (1) THE TECHNIQUE OF THE COMPARATIVE EXPERIMENT.

A. F. WATSON AND E. MELLANBY.

From the Pharmacology Department, Sheffield University.

Received for publication May 14th, 1930.

The pioneer work of Yamigawa and Itchikawa (1915) on the production of the malignant state in the skin of the rabbit's ear by means of coal-tar was followed shortly afterwards by that of Tsutsui (1918), who showed that a similar method would produce cancer of the skin in mice. This technique was used in the present investigations, which were undertaken primarily in order to explore the possibility that the initiation and development of cancerous growths might be controlled to some extent by diet. This aspect of the subject has, of course, received much attention, especially in the earlier days of experimental work on cancer, when the method employed consisted of determining the effect of certain dietetic units on the growth of transplanted tumours. More recently, Caspari (1929) and his co-workers have examined some of these effects in detail, and their papers include an extensive survey of the views of other workers in this particular field. It seemed to us, as it has to many others, that the determination of the effects of any experimentally imposed condition was more likely to lead to results when tested on the initiation and development of what might be regarded as natural neoplasms produced by tar than on the growth of transplanted tumours. It was not anticipated that any large and clear-cut effects would be obtained in the early stages of the work, since these would undoubtedly have been recognized by earlier investigators. It was considered more likely that, working under the conditions chosen, small but unequivocal differences might be observed in the development of the tumours, and it was hoped that, once obtained, it might be possible to simplify the conditions, and thus track down the factor or factors affecting the cancerous reaction. Now it is well known that the production of tar cancer in mice involves a technique which may be so crude as to conceal small but real differences. Many animals die as the result of a toxæmia set up by the tar, while the very nature of feeding experiments introduces conditions which must of necessity depress on occasions the health of experimental animals. This latter fact in investigations of this sort is of special importance. Any conditions leading to ill-health and starvation will also tend to depress the growth of cancerous tumours—a fact which most of the earlier workers have recognized. In view of these facts it seemed essential that the technique employed should be such that small differences produced by altered conditions could be easily observed, and that as a rule results should not be regarded as satisfactory and specific unless obtained under conditions when the general health of the animals in the different groups was comparable. In the present paper, therefore, such a technique, which it was hoped would expose any small differences which might exist in the relative effects of different experimental conditions on the initiation and growth of tar tumours, has been described. Since there is no such thing as a standard tar or other carcinogenic agent for experiments of this nature, it is clear that such experiments must be controlled by including a group of animals receiving a standard treatment as closely comparable as possible in each series. By this means not only can the immediate effect of any imposed change of conditions be interpreted in terms of the control group, but comparison of the control groups in each series will give some indication of the variations in susceptibility of the different series in their reaction to the carcinogenic agent.

It may be of value, first, to consider the various ways in which the activities of the tar may be asserted under ordinary conditions. Suppose we take the simple case where, say, one hundred mice are treated twice weekly for a period of four months in the interscapular region with an active sample of coal-tar, and then left for twelve months without further treatment. The observations of numerous investigators have shown that the effects of the tar are manifested in at least five well-defined ways:

(1) A certain number of mice will die as the result of the toxæmia set up by the tar.

(2) After some weeks, the period depending on the technique of treatment and the potency of the tar, small tumours* will appear at the site of tarring in a certain number of animals. The numbers of tumour-bearing animals will gradually increase for some months, irrespective of the fact that the tar treatment may have been discontinued.

(3) There will be a general tendency on the part of the majority of the tumours to exhibit slow but steady growth and to infiltrate the surrounding normal tissues.

(4) The malignant tumours as soon as they have become established will show a tendency to metastasize in the lungs or the lymph-nodes, or much less frequently in other organs. These metastatic deposits will exhibit steady growth, and provided a sufficient time has elapsed since the first deposition of the malignant cells, will in the case of the lung, assume the characteristic nodule appearance which is recognizable at autopsy.

(5) During the course of the experiment, both the animals with skin-tumours and those without will show a high incidence of epithelial tumours of the lung, which will assume the form of pearly white nodules, indistinguishable to the naked eye from the true metastatic deposits of the primary skin-tumour. Their characteristic appearance under the microscope, however, will in most cases afford a ready means of distinguishing the two types of nodules (Murphy and Sturm, 1925).

---

* The terms "papilloma" and "malignant tumour" have been reserved in the present paper for a microscopically established benign or malignant tumour respectively. The term "tumour" has been employed in the general sense to mean either a papilloma or a malignant tumour which has not yet been definitely diagnosed.

The toxæmia set up by the tar does not at first sight appear to have any direct association with carcinogenic activities displayed later in the experimental period. Since, however, the relative importance of systemic factors as compared with purely local ones in the genesis of cancer is not yet satisfactorily established, this toxæmia, induced by the tar and presumably having an important effect on the general health and condition of the animals, could not be neglected in comparative experiments such as it was proposed to carry out. Any experimental condition which may be found to influence one of these five effects may not, of course, necessarily exert a similar influence on the others. The search for an experimental treatment, for instance, which will accelerate or retard the appearance of primary tar tumours, may be fundamentally a different problem from a search for those conditions which will similarly affect the development of these tumours once they are initiated. A satisfactory method for investigating the influence of any experimental treatment on the various effects of the tar must not neglect the possibility that the conditions controlling the five ways in which these effects manifest themselves may be different in each case. At the same time there is a possibility that such differences might be quantitative rather than qualitative, and that whereas, for instance, a powerful stimulus might be necessary to suppress the production or progress of a primary skin tumour, a less powerful one of the same type might suppress the activities of such a tumour by limiting the spread of its metastases or the incidence of the indirectly associated epithelial lung tumours. If such a stimulus could be found, there would appear to be a chance that by increasing its force or applying it in a more effective way, the other carcinogenic activities of the tar might be brought under experimental control.

METHODS.

The general scheme employed involves a series of similarly controlled experiments, an experiment consisting of usually not more than five groups, each of about 100 mice (although occasionally less are used). One group of animals in each experiment is maintained on the control diet, and invariably treated in the same way regardless of which experiment it forms a part. The other groups of the experiments are subjected to varying types of treatment, and this may involve varying the dietary conditions, the inoculation of different fluids and so forth, care being taken to vary only one factor at a time.

The animals used for the experiments are selected preferably from young laboratory-bred mice and are kept in boxes, ten boxes per group. To decrease the variation due to the ages of the animals, etc., the groups are built up in such a way that the average age of the mice in one group is the same as that in another. No mice less than three months old are included. At this stage of the experiment each box contains fourteen mice, which are allowed to live together under these conditions for about a fortnight. At the end of this time the hair from a small area between the shoulders of each animal is removed by means of warm 8% sodium sulphide. Three days after the epilations the animals are examined to ensure that at least twelve mice survive in each box. The applications of tar are now commenced, and each mouse treated by means of a camel-hair brush with tar in a small (approximately ⅜ in. diameter) circular area in the centre of the epilated site. Each animal is removed from the box for this purpose. It is of importance to make the treatment with tar as uniform as possible throughout the groups. To avoid any question of the fatigue of the operator interfering with the ultimate uniformity of treatment of all groups, the latter are arranged vertically and special precautions taken with regard to order of selection for tar treatment. The boxes are selected horizontally from the first box of the first group for the first tarring of the week, and horizontally in the reverse direction (*i. e.* starting from the last box of the last group) for the second tarring and so on. Treatment with tar in this way is carried out at bi-weekly intervals for, usually, not longer than four months. There is evidence to suggest that three or even two months' treatment under these conditions is more likely to expose differences in the reaction of the experimental animals than a longer treatment. For similar reasons a tar of low carcinogenic potency seems preferable, since a highly potent agent will be more likely to mask any possible resistance created by the imposed experimental conditions.

A certain number of mice in each box will probably have died as the result of the epilations or first tarring, and the day after the first treatment with tar any mice in each box in excess of ten are withdrawn and the experiment now consists of, say, five groups, each group consisting of ten boxes of ten mice each, alive 24 hours after the first application of tar.

The control group receives no other treatment, and is maintained under exactly the same conditions of diet, etc., as the corresponding control group in each of the preceding (and following) experiments of the series. Each of the other groups receives a special type of treatment either throughout the whole experimental period or else for a more restricted time. If this treatment involves a variation of diet, the animals are maintained on the special diet for two or three weeks prior to the first application of tar. A control diet consisting of powdered bread (4 parts), Sussex ground oats (1 part) and cabbage (1 part), mixed in an electric mixer and made into a uniformly moist paste with water, fed daily and supplemented once per week with cod-liver oil (0·25 c.c. per mouse) and marmite (0·25 gm. per mouse), has been used throughout the present series of experiments for the control group.

Each animal is given a number, and the first appearance of a tumour is recorded by an experienced observer. Separate columns are kept in the note-book for recording the deaths of tumour-bearing and tumour-free animals. Each animal as it develops a tumour is segregated into one compartment of a three-compartment box of the same type and size as those of the main groups. All the animals are examined once per fortnight, the shape and size of the tumours being recorded in a chart-book. Any mice which have developed tumours since the previous inspection are segregated in the special three-compartment boxes.

Except in unusual circumstances no animals are killed during the experiment, and no efforts are made to establish malignancy by methods such as removal of fragments of tumour tissue for microscopical examination before death or by autoplastic inoculation of the primary tumour, etc. In this way the experimental conditions, the effects of which are under survey, are in no way modified or altered throughout the whole period. All the animals that die at any time after the cessation of tarring are subjected to post-mortem examinations. The organs and an extensive area of skin from the interscapular region, together, if present, with a portion of tumour-tissue, are removed and transferred to formol saline for future examination. At the end of a definite 30-day period (240–480 days) after the commencement of the experiment, all the animals alive in the different groups are killed and a detailed post-mortem examination carried out.

ARRANGEMENT OF RESULTS.

It now becomes necessary to arrange the data accumulated throughout the experimental period in order to find whether the imposed conditions have exerted any influence on the general course of the activities of the tar, as illustrated by the behaviour of the control group common to each experiment. As previously stated, the effects of the tar are exerted in at least five well-defined ways, and it will be necessary to explore each of these in order to give an answer to the main question. The methods which have been employed for utilizing the data may perhaps be illustrated by a special case. Five groups each of ninety-five mice were subjected to applications of tar twice weekly over a period of four months (120 days) and then left for a further period of 360 days, during which time they were kept under careful observation. One group, F, was maintained on the control diet described above. In the other groups, A, B, C and D, the experimental conditions consisted of modifications of the diet, in each group, an aliquot part of the basal diet being substituted by a different foodstuff of animal origin. No other factors were varied during the 480 days of the experiment.

By referring the dates of the appearance of the tumours and the deaths of the animals to a zero date (day of first tarring) and the simple re-arrangement of the data obtained, the following table can be constructed :

Table I.—*Data Collected during Course of Experiment lasting 480 Days.*

Initial number of mice in each group = 95.

Period of experiment (days).	Column.														
	1. Number of mice dying without tumours.					2. Number of mice dying with tumours.					3. Incidence of tumours.				
	A.	B.	C.	D.	F.	A.	B.	C.	D.	F.	A.	B.	C.	D.	F.
1– 30	12	13	9	13	14	0	0	0	0	0	0	0	0	0	0
31– 60	4	1	10	3	2	0	0	0	0	0	0	0	0	0	0
61– 90	6	3	1	4	1	0	0	0	0	0	0	0	1	2	3
91–120	2	3	1	0	1	0	0	1	0	0	12	5	6	7	6
121–150	1	0	1	0	3	0	1	0	0	1	8	14	5	10	9
151–180	2	4	4	2	1	0	1	0	1	1	16	14	14	19	17
181–210	4	4	5	0	0	4	4	2	3	2	0	5	4	5	10
211–240	2	1	1	1	0	2	5	1	3	1	7	10	5	6	4
241–270	0	2	7	3	5	7	11	16	6	8	2	1	5	1	3
271–300	1	1	2	1	6	13	7	8	7	13	1	3	0	4	1
301–330	1	5	0	3	0	8	12	6	17	8	1	1	2	1	2
331–360	0	1	5	1	1	7	3	1	6	8	2	0	0	1	2
361–390	1	1	0	2	1	3	1	1	6	8	1	0	1	0	0
391–420	3	0	5	0	1	1	4	1	5	4	0	1	1	0	1
421–450	2	0	0	2	0	2	2	2	1	2	2	0	0	0	0
451–480	2	1	0	1	0	2	3	0	1	1	0	0	0	0	0
Totals	43	40	51	36	36	49	54	39	56	57	52	54	44	56	58

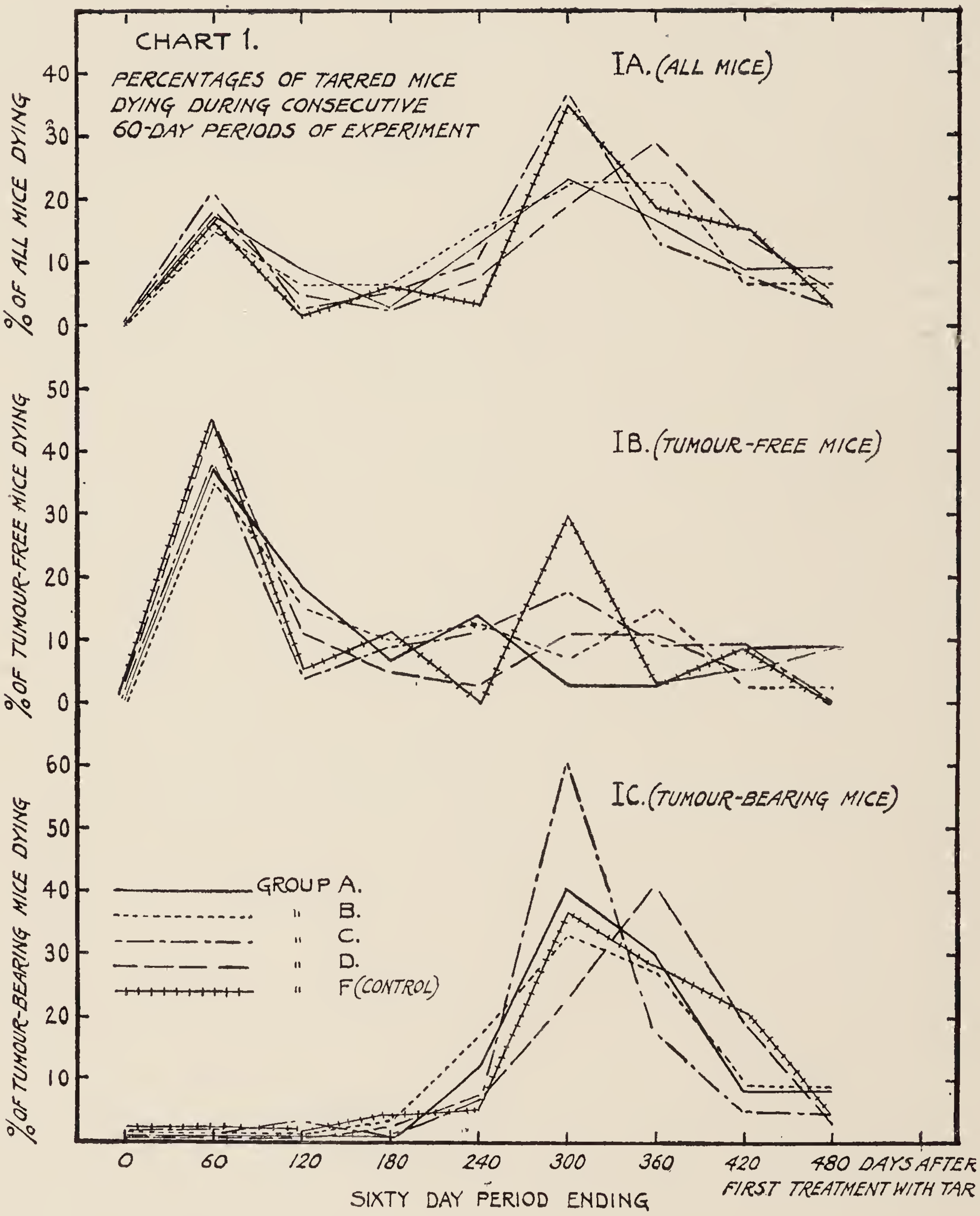
	A.	B.	C.	D.	F.
Number of tumour-bearing mice alive after 480 days	3	0	5	0	1
,, tumour-free mice alive after 480 days	0	1	0	3	1

Treatment with tar was discontinued after the 120th day.

It will be seen that the greatest incidence of tumours and the greatest number of deaths in the tumour-free and the tumour-bearing mice occur within fairly well-defined zones. The data from such a table can be utilized in various ways, and with other information obtained during the course of the experiment form a useful basis for assessing the influence of the particular experimental condition on the various effects of tar on the animals.

(1) *The Relative Resistance of the Mice in each Group to the Toxic Effects of the Tar, and the Span of Life of* (a) *all Animals,* (b) *Tumour-free Animals, and* (c) *Tumour-bearing Animals.*

The data from Table I can be used to construct Chart 1 where the percentages of (*a*) all animals, (*b*) tumour-free animals and (*c*) tumour-bearing animals dying during consecutive 60-day periods of the 480 days of the experiment are shown.



The curves in the chart exhibit two series of maxima, one in the early part of the experiment (during the first 60-day period) as a result of the toxæmia set up by the tar, and the second during the period 240–360 days after the first treatment with tar, when the animals with skin-tumours die in greatest numbers. No clear-cut differences in the reaction of the mice on the different diets to the tar appear to exist, although the tumour-bearing animals of Group D show a tendency to live longer than those of the other groups. These facts are clearly indicated in the three series of curves in the chart.

The average spans of life of the mice in the five groups after the first application of tar are shown in Table II :

TABLE II.—*The Average Span of Life in Days of the Mice in each of the Five Experimental Groups after the First Treatment with Tar.*

	Group.				
	A.	B.	C.	D.	F.
Total number of all mice dead by the 480th day	92	94	90	92	93
Average span of life (days)	236	234	221	251	247
Number of tumour-bearing mice dead by the 480th day	49	54	39	56	57
Average span of life (days)	307	298	281	312	314
Number of tumour-free mice dead by the 480th day	43	40	51	36	36
Average span of life (days)	155	147	174	157	143
Number of mice alive at the 120th day (*i. e.* at the end of the tar treatment), and which died before the 480th day without developing skin-tumours	19	20	30	16	18
Average span of life (days)	297	259	269	313	258

The main feature of interest is that the tumour-bearing animals have had practically twice the life-span of the other mice, and in four cases out of five actually lived longer (despite the skin-tumours), than the mice which were alive at the end of tar treatment (120th day) and subsequently never developed skin-tumours.

(2) *The Relative Resistance of the Mice in each Group to the Induction of Skin-Tumours during the 480 Days of the Experiment.*

Since it is obvious that the death-rates of the mice will vary appreciably according to the severity of the experimental treatment (in the present case, of course, according to the dietetic treatment), tables such as Table I, column 3, illustrating the mere incidence of tumours will be of little use for comparative purposes. Two methods have been used to avoid this difficulty.

(a) *The Percentages of the Animals Surviving at any Period with Tumours.*

Most authors have surmounted the difficulty by constructing graphs showing the relative percentages of the total number of animals alive at any period of the experiment with tumours. Table III shows the necessary re-arrangement of data from Table I for this purpose :

Table III.—*Re-arrangement of the Data from Table I for the Determination of the Percentages of the Surviving Mice bearing Tumours at the end of Consecutive 30-Day Periods of the Experiment.*

Initial number of mice in each group = 95.

Period ending, day.	Column.														
	1. Total surviving mice.					2. Mice alive with tumours.					3. $\% \left\{ \frac{\text{Tumour-bearing mice.}}{\text{Total surviving mice.}} \right.$				
	A.	B.	C.	D.	F.	A.	B.	C.	D.	F.	A.	B.	C.	D.	F.
30th	83	82	86	82	81	0	0	0	0	0	0	0	0	0	0
60th	79	81	76	79	79	0	0	0	0	0	0	0	0	0	0
90th	73	78	75	75	78	0	0	1	2	3	0	0	1·3	2·7	3·8
120th	71	75	73	75	77	12	5	6	9	9	16·9	6·7	8·2	12·0	11·7
150th	70	74	72	75	73	20	18	11	19	17	28·6	24·3	15·3	25·3	23·3
180th	68	69	68	72	71	36	31	25	37	33	52·9	44·9	36·8	51·4	46·5
210th	60	61	61	69	69	32	32	27	39	41	53·3	52·5	44·3	56·5	59·4
240th	56	55	59	65	68	37	37	31	42	44	66·1	67·3	52·5	64·6	64·7
270th	49	42	36	56	55	32	27	20	37	39	65·3	64·3	55·6	66·1	70·9
300th	35	34	26	48	36	20	23	12	34	27	57·1	67·6	46·1	70·8	75·0
330th	26	17	20	28	28	13	12	8	18	21	50·0	70·6	40·0	64·3	75·0
360th	19	13	14	21	19	8	9	7	13	15	42·1	69·2	50·0	61·9	78·9
390th	15	11	13	13	10	6	8	7	7	7	40·0	72·7	53·9	53·9	70·0
420th	11	7	7	8	5	5	5	7	2	4	45·5	71·4	100·0	25·0	80·0
450th	7	5	5	5	3										
480th	3	1	5	3	2										

Treatment with tar was discontinued after the 120th day.

The total surviving mice in each group at the end of any 30-day period can be obtained from Table I by determining the number of tumour-free mice (column 1) and tumour-bearing mice (column 2) dead before the end of the particular 30-day period and subtracting the sum from 95, the total number of mice in each group at the commencement of the experiment.

The number of mice alive with tumours at the end of any 30-day period can be obtained from Table I by determining the number of mice that have developed tumours before the end of the particular 30-day period (column 3), and subtracting from this total any tumour-bearing animals that may have died previously (column 2).

Thus by the end of the 270th day in the control group F, for instance, 27 tumour-free and 13 tumour-bearing mice had died, so that 95 — 40 = 55 animals remained. 52 mice had developed skin-tumours (column 3) before the end of the same day, and of these, 13 (column 2) had died, leaving 39 mice with skin-tumours on the 270th day. The percentage of the surviving mice with skin-tumours on this day was, therefore, $\frac{39 \times 100}{55} = 70{\cdot}9$.

A few tumours appear in groups C, D and F during the 30-day period ending the 90th day, and henceforward the percentages of the surviving mice with skin-tumours increase in all groups until about the 270–300th-day period when there is a tendency for the percentages to decrease somewhat. During the critical periods of the experiment (90–330th) day the percentages of the surviving mice with skin-tumours is low in group C compared with the other groups, and there is a suggestion here that the experimental treatment of this group is to a slight extent antagonizing the activity of the tar as manifested by the appearance of skin-tumours.

(b) *The Percentage Liability of the Animals Surviving without Skin-Tumours at the end of Consecutive 60-day Periods to Develop such Tumours during the following 60 Days. The Determination of the Group Experimental Condition Indices.*

A method which has certain advantages over the one described above for determining the relative resistance of the mice in each group to the induction of skin-tumours, consists of an examination of curves showing the relative percentage liabilities of the tumour-free mice to develop tumours in a given time. Table IV shows the necessary re-arrangement of data from Tables I and III for this purpose :

TABLE IV.—*Re-arrangement of the Data from Tables I and III for the Determination of the Percentage Liability of the Mice Surviving and without Tumours at the end of each 60-Day Period to become Tumour-Bearing in the following 60 Days.*

Initial number of mice in each group = 95.

	Column.														
Period ending, day.	1. Mice surviving without tumours.					2. Incidence of tumours during following 60 days.					3. Percentage liability of the mice to become tumour-bearing in the following 60 days.				
	A.	B.	C.	D.	F.	A.	B.	C.	D.	F.	A.	B.	C.	D.	F.
60th	79	81	76	79	79	12	5	7	9	9	15·2	6·2	9·2	11·4	11·4
120th	59	70	67	66	68	24	28	19	29	26	40·7	40·0	28·4	43·9	38·2
180th	32	38	43	35	38	7	15	9	11	14	21·9	39·5	20·9	31·4	36·8
240th	19	18	28	23	24	3	4	5	5	4	15·8	22·2	17·8	21·7	16·7
Average percentage liabilities of the animals to develop tumours on the 60th, 120th, 180th and 240th days											23·4	26·9	19·1	27·1	25·8

By referring the value of the control group (F) to 100, the ratio of these average percentage liabilities of the animals to develop tumours becomes 91 : 104 : 74 : 105 : 100 for groups A, B, C, D and F respectively.

The total number of mice in each group surviving without skin-tumours at the end of any 60-day period can be obtained from Table III by subtracting the number of mice alive with tumours (column 2) from the total number of surviving mice (column 1).

The incidence of skin-tumours in the tumour-free mice alive at the end of any 60-day period during the following 60 days can be obtained from Table I (column 3).

Thus from Table III it will be seen that there were, on the 120th day in the control group F for instance, 77 mice alive (column 1), and of these, 9 had tumours (column 2). There were, therefore 68 mice without skin-tumours. During the following 60 days (*i.e.* during the period 120–180 days after the first tar treatment) 26 of these animals developed skin-tumours (Table I, column 3). The percentage liability of the mice surviving without skin-tumours on the 120th day to develop such tumours in the following 60 days was, therefore $\frac{26 \times 100}{68} = 38{\cdot}2$.

The results for all groups for the 60th, 120th, 180th and 240th days are expressed graphically in Chart 2.

The percentage liabilities of the animals to develop tumours show a maximum in all groups on the 120th day, which is equivalent to saying that the tumours appear in relatively larger numbers in all groups in the 60 days following the cessation of tar treatment. The curve for Group C occupies an abnormal position compared with those of the other groups.

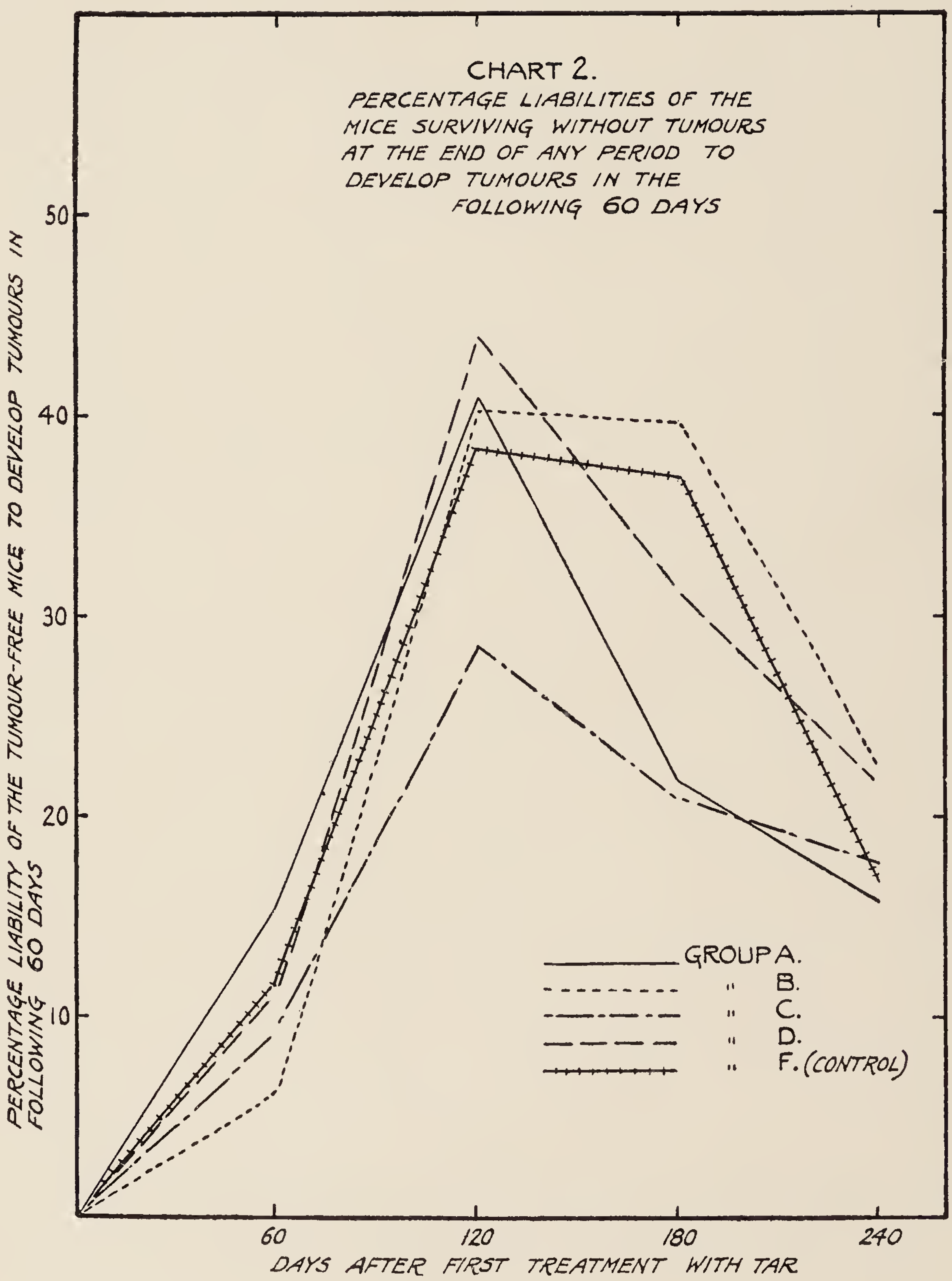


CHART 2.
PERCENTAGE LIABILITIES OF THE MICE SURVIVING WITHOUT TUMOURS AT THE END OF ANY PERIOD TO DEVELOP TUMOURS IN THE FOLLOWING 60 DAYS

This second method for comparing the resistance of the mice to the induction of tumours in the different experimental groups is not so limited in its application as the first method. It has occasionally proved useful in cases where the effects of one type of experimental treatment on the incidence of skin-tumours and a quite different treatment on the rate of growth or metastatic spread of the developing tumours is being examined. In such a case as this the first method of utilizing the curves illustrating the percentages of the surviving animals with tumours in each group for comparative purposes cannot be employed, since two treatments, one before and one after the appearance of the tumours, are involved. The second method suggested, on the other hand, is not concerned with the history of the tumour-bearing animals after the first appearance of the tumour (*i. e.* when a different treatment might be commenced), and can therefore be employed with safety to estimate the comparative reactions of the animals in the different groups to the effects of the tar.

The second method can also be adapted to express the relative influence of a series of experimental treatments compared with that of an untreated control group, as a definite numerical ratio. In the present experiment, for instance, the relative influence of the four diets on the induction of tar tumours in the animals may be compared with that of the control group by determining the average of the percentage liabilities of the tumour-free mice, say, on the 60, 120, 180 and 240th days to develop tumours in the 60-day periods respectively following these selected days. It will be seen from Table IV that these average percentage liabilities of the animals to develop tumours is 23·4, 26·9, 19·1, 27·1 and 25·8 for groups A, B, C, D and F respectively. If we assign the value 100 to the control group (F), then the ratio of the percentage liabilities of the animals to develop tumours becomes 91 : 104 : 74 : 105 : 100 for the five groups. These group values may be conveniently termed the *experimental condition indices (E.C.I).* With the exception of that for the C group the E.C.I. values are very similar and, broadly interpreted, this may be taken as suggesting that the activity of the tar as manifested by the appearance of skin-tumours is the same for groups A, B, D and F but slightly suppressed in group C.

(3) *The Relative Rates of Growth of the Tumours in the Different Groups from the Time of their First Appearance until the Death of the Animal; the Periods of Survival of the Animals after the First Appearance of the Tumour.*

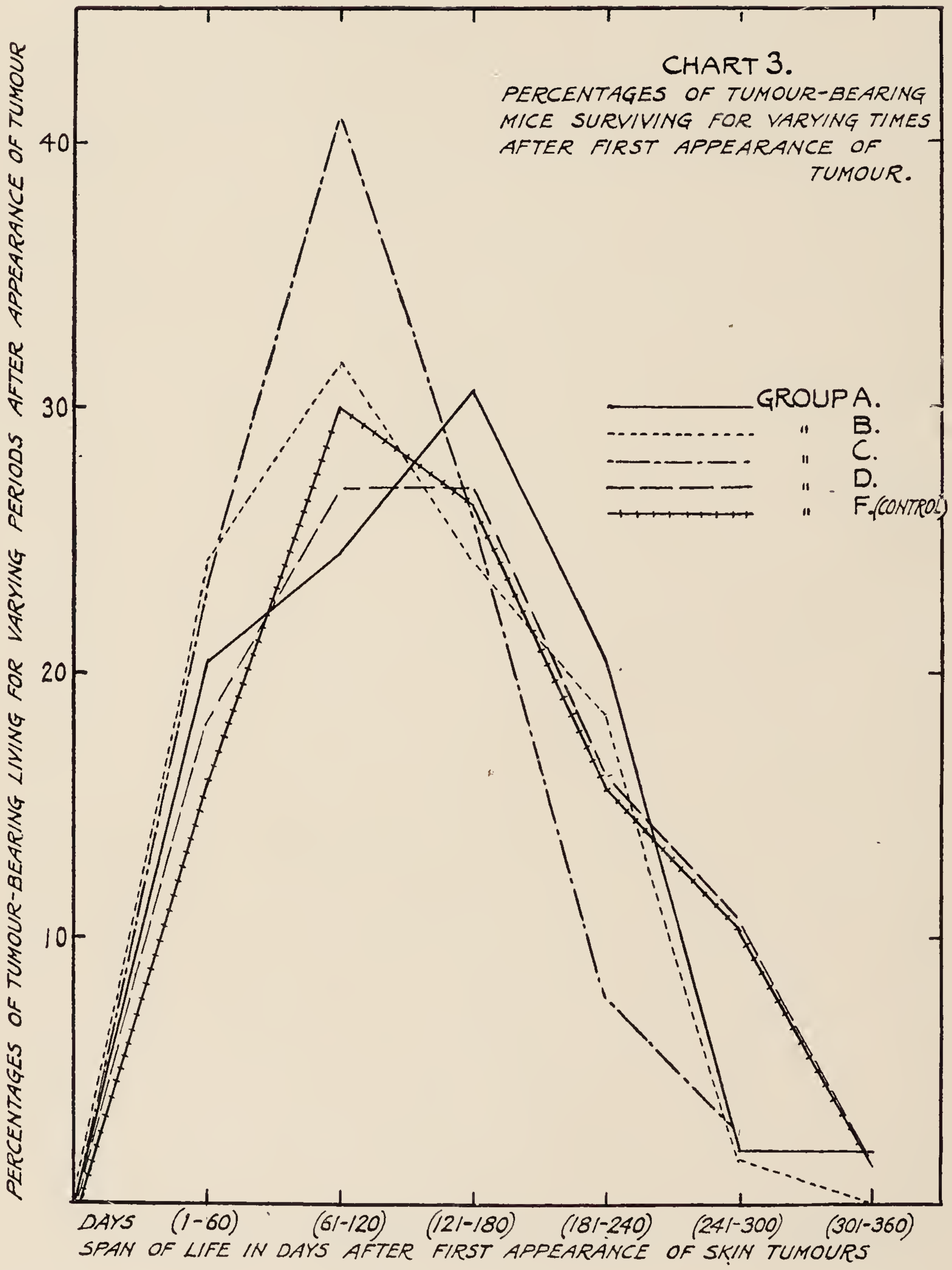
A general impression of the shape, form and rate of growth of the skin-tumours in each group can be obtained from the fortnightly chartings over the experimental period. No evidence existed in the present series to suggest that the rates of growth had in any way been influenced by the experimental conditions.

The following table shows the average period in days which the tumour-bearing animals in each group lived after the first appearance of the skin-tumour :

Group.	A.	B.	C.	D.	F.
Number of tumour-bearing mice dead by the 480th day after the first treatment with tar	49	54	39	56	57
Average period of survival in days of the mice after the first appearance of the skin-tumour	130	118	105	137	135

As a matter of general interest, the average period of survival of the 255 mice of all groups, after the appearance of the tumours, was 126·5 days. It will be observed that the animals of group C survived for a somewhat shorter period of time after the appearance of the tumours than those of any other groups.

These survival results may be presented in a slightly different form,



depending on the percentages of the tumour-bearing animals surviving for different 60-day periods after the first appearance of the tumours. Such a presentation is shown in Chart 3.

There was a general tendency for the animals of group A to survive for a longer period than those of other groups, where 61–120 days was the most frequent period for the animals to survive after the appearance of the tumour.

(4) *The Occurrence of Metastatic Deposits in Other Organs and Tissues, more especially the Lungs, and the Incidence of Primary Epithelial Lung Tumours in the Animals of the Five Experimental Groups.*

Metastases to the primary skin-tumour occur frequently in mice tarred under the conditions described. They are found more especially in the adjacent lymph-nodes and in the lungs, and less frequently and at a later period of the experiment in the liver, spleen, heart, etc. Table V shows the results of the examination of 257 tumour-bearing mice from the five groups of the experiment. For the purpose of the examinations, the organs of each animal were scrutinized by two observers at the time of autopsy. The lungs from each animal were again examined after they had been fixed in 10 % formol saline for 48 hours. This preliminary examination was followed up by a microscopical survey of sections of the skin at the site of tarring, of nodules of the lung, and of any tumour-like deposits in other organs and tissues.

TABLE V.—*Summary of Post-mortem Examinations of Tumour-Bearing Mice*

	Column.								
Group.	1. Total numbers of tumour-bearing mice.			2. Presence of nodules in lung tissues.				3. Presence of tumour-like deposits in other organs. Visible at autopsy.	
				Visible at autopsy.		Visible after fixation in 10% formol saline.			
	Died.	Killed.	Examined.*	No.	% total.	No.	% total.	No.	% total.
A	49	3	51	17	33·3	23	45·1	10	19·6
B	54	0	54	17	31·5	29	53·7	5	9·3
C	39	5	42	11	26·2	21	50·0	3	7·1
D	56	0	54	18	33·3	30	55·6	8	14·8
F	57	1	56	20	35·7	32	57·1	11	19·6
Totals	255	9	257	83	32·7	135	52·4	37	14·4

* Of the 264 post-mortem examinations, the findings of 1 from Group A, 2 from C, 2 from D and 2 from F have not been included.

The percentages of the animals with lung nodules distinguishable at autopsy varied in the different groups from 26·2 to 35·7, with an average for all mice of 32·7. The percentages of animals with tumour-like deposits distinguishable at autopsy in organs other than the lungs varied in the five groups from 7·1 to 19·6, with an average for all mice of 14·4. It is obvious, therefore, that these nodules of the lungs are a more consistent feature of the post-mortem examinations than the cancerous deposits in other organs.

After the fixation of the lung tissues in formol saline for 48 hours, many nodules which were indistinguishable before become readily recognizable to the naked eye. Thus the percentages of the animals with lung nodules which were visible to the naked eye after such fixation of the tissues varied in different groups of the present experiment from 45·1 to 57·1, with an average for all mice of 52·4. To summarize, nodules were distinguishable in the lungs of approximately one in every three tumour-bearing animals at post-mortem examination, and after fixation in formol saline, in the lungs of approximately one in every two such animals. A proportion of these lung nodules in any group of tarred mice almost invariably escapes the closest examination until after the fixation of the tissues in formol saline. Even then, of course, some may still remain undetected until the lungs have been subjected to such a technique as that adopted by Fibiger and Møller (1927), who carried out an exhaustive examination of the tissues by means of serial sections. At the same time, in comparative experiments where the general health and weight curves of the animals in the different groups are similar, data on the percentage incidence of these lung nodules visible to the naked eye after fixation of the tissues should prove useful criteria of the relative influence of the experimental conditions. The exact nature of these nodules cannot, of course, be decided until microscopical examinations have been carried out. The histology of the lung tissues of the animals of the present experiment has been studied in detail, and the general results are similar to those published by Fibiger and Møller (*loc. cit.*). In the majority of cases the lung nodules of the mice with skin-tumours consist of squamous epitheliomata, secondary to the primary tumour, or primary epithelial lung tumours of an adenomatous type. In the tarred animals without skin-tumours, the lung nodules are normally of the latter type. The incidence of this type of lung nodule visible to the naked eye after fixation of the tissues in formol saline in normal untarred mice of the same breeding stock was found to be 6%, of which 4% were visible at autopsy. The susceptibility to this type of lung tumour is, in some way not yet understood, increased considerably by treating the skin of the mice with tar, whether skin-tumours occur or not. That such an increased susceptibility, experimentally produced, can be inherited by the offspring of the tarred mice is suggested by the recent work of Schabad (1929). The problem of lung tumours in mice, although a complicated one, is obviously of importance. No significant differences between the histological picture of the lung nodules of the animals in the five groups of the present comparative experiment could be traced.

SUMMARY.

(1) A method which has been used for the determination of possible small differences in the reaction of mice, maintained under varying experimental conditions, to the periodical applications of coal tar to the skin has been described.

(2) The influence of the experimental conditions has been assessed in an experiment lasting 480 days, by comparing the reaction of the mice to the tar in regard to the following points with that of a group of similarly tarred but otherwise untreated control mice.

(a) *The induction of skin-tumours as measured by one of the following methods:*

(1) The examination of the percentage numbers of surviving mice bearing skin-tumours at the end of each 30-day period of the experiment.

(2) The determination of the percentage liabilities of the mice alive without skin-tumours at the end of each 60-day period to develop such tumours in the following 60 days.

(3) The determination of the so-termed experimental condition index (E.C.I.) by the method described.

(b) *The rate of growth of the skin-tumours from the time of their first appearance until the death of the animal.*

(c) *The percentage number of tumour-bearing mice with typical nodules of the lung visible after fixation of the tissues for 48 hours in 10% formol saline.*

(d) *The microscopical structure of the primary skin-tumour, its metastases in the lungs and other tissues, and of the epithelial tumours of the lung.*

(3) Of 255 mice with skin-tumours, 52·4% were found to contain typical nodules of the lung distinguishable after fixation of the lung tissues for 48 hours in formol saline. Nodules of the lung in only 32·7% of the animals were visible to the naked eye before such fixation of the tissues.

We are indebted to the Yorkshire Cancer Research Council for the expenses of and means for carrying out this investigation, and to Prof. P. J. Daniell, of Sheffield University, for his help and criticism of the statistical data.

One of us (A. F. W.) also wishes to express his thanks to the University of London for the Laura de Saliceto Research Studentship, which was held during the course of the work.

REFERENCES.

Caspari, W., Ottensooser, F., Fauser, M , and Blothner, E.—(1929) *Z. KrebsForsch.*, **29**, 334.

Fibiger, J., and Møller, P.—(1927) *K. danske Vidensk. Selsk. Biol. Meddelelser.*, **6**, 1.

Murphy, J. B., and Sturm, E.—(1925) *J. Exp. Med.*, **42**, 693.

Schabad, L. M.—1929) *Z. KrebsForsch.*, **30**, 24.

Tsutsui, H.—(1918) *Gann.*, **12**, 17.

Yamagiwa, K., and Itchikawa, K.—(1915) *Verh. jap. path. Ges.*, VI^{te} Tagung.

ADLARD AND SON, LIMITED, LONDON AND DORKING.